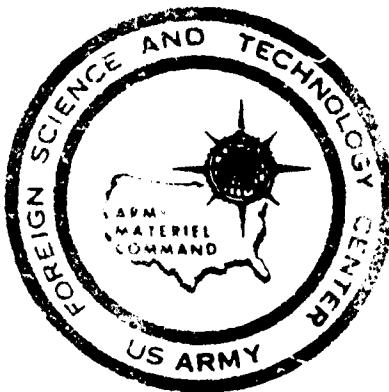


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THE STUDY OF CULTURAL MORPHOLOGICAL AND VIRULENT
PROPERTIES OF THE B. MALLEI

CHINGHISIAN MONGOLIAN PEOPLES REPUBLIC

TECHNICAL TRANSLATION

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OF THE B. MALLEI
by

Tsegmed and N. G. Ipatenko

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Ten strains of the Bacillus mallei, which made it possible for the first time to prepare under the conditions of the Mongolian Peoples Republic the first series of mallein and to introduce it into wide production practice for diagnosis of the given sickness were selected as the result of the investigations. The selected strains turned out to be active, judging by their virulence.

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THE STUDY OF CULTURAL MORPHOLOGICAL AND VIRULENT PROPERTIES OF THE B. MALLEI

Eighteen cultures from 30 horses with a positive ophtalmic probe and a complement fixation test of four plus were selected in the Glanders Laboratory of the Science Research Institute of Husbandry and Veterinary Science. Glanders knots were present in the lungs of all the animals at the time of the slaughter. Plantings were made on a glycerinized potato, MPA, MPB, and on a bullion with 4-5% of glycerine.

On the second day slight turbidity was observed in the bullion which increased on the 10th - 12th day. On the third-fourth day a greyish-white ring appeared in the test tube, and two weeks later the surface of the medium was covered by a thick film, which descended to the bottom when the test tube was shaken. The microbes grew on the agar in the form of greyish-white, semitransparent colonies of honey-amber shade.

Slow growth with the formation, after several days of a slimy greyish-white film from which slimy streaks stretched to the bottom along the walls was observed on the bullion with glycerine (pH 7.2 - 7.4).

On the glycerinized potato, a delicate, transparent slimy, amber-honey deposit appeared on the third day, and on the fifth day the culture became more mucous and acquired a dark-brown shade. Its transparency was lost and greenish or brownish pigment appeared around the colony.

All the strains curdled milk in 6-12 days without pentonization. When litmus was added, the curdled milk became reddish. The glanders cultures were checked twice for the formation of indole, ammonia, and hydro-sulfate. All the glanders cultures produced negative results as far as formation of indole and positive results as far as ammonia and hydrosulfate were concerned.

When the cultures were planted onto a variegated series (lactose, glucose, saccharose, mannitol, maltose, dulcite) acid was produced, but without gas on media with lactose and glucose (four plus). The mannitol and maltose were of three plus, saccharose and dulcite were of two plus. Out of the 18 investigated cultures only two liquified the gelatin slightly (cultures No. 005 and 006).

Three types of colonies were observed on the solid media: first - those characteristic for the glanders microbes; second - round and shapeless, yellowish ones with depressed centers (crater), with granular structure and smaller size in comparison to the first type.

An emulsion from the glanders knots of the lungs in the physiological solution of cooking salt was used as the material for the infection of cats and guinea pigs. This emulsion was steeped from three to six hours with the saturation of 1g per 3 ml. of the physiological solution (the pathological material was finely cut, powdered in a mortar beforehand and then added to the physiological solution).

Three ml. of the emulsion was injected into cats (in the nape sac area) at a depth of 3 - 4 cm. in the direction of the oblongata. An abscess was formed in the site of the injection which later opened and became an ulcer.

According to our observations, the experimental glanders in cats passes in the form of a severe septicemia. As the infection became more general ulcers appeared on different areas of the skin and were accompanied as a rule by a discharge from the nose (lung and nose forms). Then emaciation took place and the animal died in 4-6-12 days.

Into the guinea pigs, (male) 1 ml of the emulsion was introduced into the abdominal cavity in the direction of the testicles, and, when the material was contaminated by outside microflora, it was introduced subcutaneously.

When pure cultures of B. Mallei were selected and the cats infected with 1 ml of one billionth suspension made according to the optical paratyphoid standard, the cats died on 4, 6, 10, and 12 days after the infection, with the formation of glanders knots in the parenchyma of the lungs. Transplants were made from the knots onto the usual nutrient media (pH 7.2 - 7.4) with the addition of 4-5% of glycerine. Pure culture was obtained in such cases for further work and differentiation from the activator of melioidosis. (Act. Whitmore). The rabbits infected by the same culture and with the same dose remained alive.

After the subcutaneously injected infection of the guinea pigs by the B. Mallei culture with a 0.3-0.5 ml dose of the one billionth suspension made according to optical paratyphoid standard, they died on the 15th - 19th and 25th day.

On the 4th - 12th day suppurative periorchitis and orchitis developed in the males with abdominal infection, and glanders knots were formed in the parenchymatous organs.

On the 18 studied cultures of glanders activators, the microbes appeared in some cases as small straight or slightly bent and short rods with rounded ends, and in other cases they appeared as long rods somewhat like thin threads. In the smears the rods (bacilloids) were often distributed in pairs.

In the young cultures they had even contours, and in the old ones they had uneven contours. The ends of the rods can have pointed or flask like shapes, besides the rounded off form. When stained grains can be detected in large quantities.

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Figure 1. Guinea pigs No. 15 and No. 16 were infected with 0.5 ml dose of one billionth suspension of *B. Mallei* made according to the optical paratyphoid standard. They fell on the sixth day with clearly pronounced orchite clinic.

All the cultures selected by us were immobile, but certain strains had intensive molecular activity. The conducted experiments confirm the previously expressed opinion on this question of N. E. Tsvetkov, V. Z. Chernyak (1935, 1947), S. N. Vyshellesskiy (1954), B. Y. Elbert (1957), Y. E. Kolyakov (1948-1960), and Gutira and Marek (1936, 1961).

The smears were stained according to Gram, Leffer and Romanovsky-Gizma. The smears were stained very well by the 1:10 and 1:5 diluted fuxin of Ciel and Pfeiffer.

THE RESULTS OF THE *B. MALLEI* CULTURE INVESTIGATION

Serum	Dilution of Serum	The antigen of the GNKI and Songinsky Combine	Culture number							
			001	003	004	005	008	009	0010	
Reaction evaluation										
		Dilution	Reaction Evaluation							
RA according to Nobel										
Positive	1:10	1:1	++++	++++	++++	++++	++++	++++	++++	++++
	1:20	1:1	++++	++++	++++	++++	++++	++++	++++	++++
	1:50	1:1	++++	++++	++++	++++	++++	++++	++++	++++
RA in test tubes										
Positive	1:20	1:1	++	+	+++	++++	+++	++++	++++	++++
	1:50	1:2	++	+	+++	++++	++++	++++	++++	+++
RA on the slides (glass slips)										
Positive	1:20	1:1	+++	++	++++	+++	++++	++	+++	+++
	1:50	1:1	+++	++	+++	+++	++++	++++	+++	+++

Note: The dilution of the cultures of the *B. Mallei* with the negative serum and physiological solution was 1:1. The reactions with the negative serum and physiological solution were negative in all cases. The agglutination reactions made according to Nobel (in test tubes and on the slides) of the positive serum in the mentioned dilutions with cultures 002, 006, and 007 were evaluated as being four plus.

The mentioned cultures were investigated in the laboratory with the standard antigen, positive and negative GMKI serum and they compared with the standard strain of the GMKI 5584, as well as with the antigen, and positive and negative serums made by Songinsky combine. The cultures were checked according to Nobel, and the reactions of agglutination were checked in test tubes and on the slides (table).

After the cultures were checked for their cultural, morphological and virulent properties as well as appurtenance to the *B. Mallei*, nine series of antigen were prepared, which produced in the complement fixation test with positive and negative serums in a titre of 1/100 a positive reaction of three and four plus. Then 9 strains were selected for the preparation of the locally made mallein.

The first mallein series of local strains of the *B. Mallei* were tried out on 12 sick horses. At the same time 2 - 3 drops of standard Soviet mallein were introduced into one eye, and the investigated mallein into the other eye, and 0.2 ml of standard mallei were injected into the other side. The test was successful, and the locally made mallein received high evaluation.

After this the mallein was checked by commission on 100 horses, among which 50 did not react to the mallein and 50 reacted to mallein ("malleinites").

The tested malleins were diluted before introduction by a sterile distilled water in the following proportions: 8:2; 6:4; 4:6; 2:8. The standard mallein was not diluted for the horses which did not react to mallein, only undiluted investigated mallein was introduced into one eye. The horses which reacted to mallein were divided into five groups (ten in each group). On the animals of the first group, undiluted mallein was used, and to five horses it was introduced into the left and to five into the right eye.



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A horse with a positive ophthalmoprobe and complement fixation test. Strain No. 22265 used for the preparation of mallein was obtained from this horse.

At the same time standard mallein was introduced into the other eye. The rest of the animals received diluted mallein in the same way.

The results of the reactions were read after 3, 6, 9, 12, and 24 hours. The reactions were evaluated by pluses:

Four plus denotes sharply pronounced conjunctivitis (profusion of pus excretion and edema of the conjunctiva).

Three plus denotes conjunctivitis with pus running from the inner edge of the eye, but the edema of the conjunctiva was somewhat smaller.

Two plus denotes edema of the conjunctiva and pus inside the conjunctival sac.

One plus denotes the reddening of the conjunctiva and watering of the eye.

In healthy horses the reaction to the introduction of the investigate mallein was negative.

CONCLUSIONS

1. Ten strains of the B. Mallei, which made it possible for the first time to prepare under the conditions of the Mongolian Peoples Republic the first series of mallein and to introduce it into wide production practice for diagnostics of the given sickness, were selected as the result of the investigations.
2. The selected strains turned out to be active judging by their virulence.

THE WOMEN VETERINARY SPECIALISTS OF THE GERMAN PEOPLES REPUBLIC

More and more women work in the German Peoples Republic carrying out responsible veterinary work, which several years ago was considered to be only for men. Eighty-five women veterinarians are directors of Government veterinary hospitals. Among the 15 regional veterinary doctors, two are women.

The veterinary clinic of Potsdam is one of the modern establishments in the German Peoples Republic. Here are treated the long horned cattle, horses, and hogs; there is, also, a section for small animals.

The picture shows Dr. Pringel (on the right) and assistant Pakazch conducting a surgical operation on the rear extremity of a pure bred bull.

photo by ADN - TASS

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